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Acute Lethality of Wastewater Disinfection Alternatives to Juvenile Rainbow Trout

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ACUTE LETHALITY OF WASTEWATER DISINFECTION ALTERNATIVES TO JUVENILE RAINBOW TROUT (SALMO GAIRDNERI)

by

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RESEARCH PROGRAM FOR THE ABATEMENT
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ABSTRACT

Increased concern over the environmental hazards arising from chlorination of municipal wastewaters prompted an investigation into the acute lethality of viable disinfection alternatives. Ninety-six-hour, continuous flow, rainbow trout bioassays were used to compare the acute lethality of secondary treated municipal wastewater before and after disinfection with chlorine, chlorine dioxide, ozone and ultraviolet light. Dechlorination was accomplished by the addition of sodium sulphite or 24 hours storage.

During the eight-month study, non-disinfected, municipal wastewater was non-acutely lethal in 15 of 17 trials. Similarly, wastewater disinfected with ozone was non-acutely lethal in five of six trials. The mortality in both the non-disinfected and ozonated wastewaters could be directly related to mechanical upsets in the treatment systems. Wastewater disinfected with ultraviolet light was also non-acutely lethal.

Ninety-six-hour LC_{50}'s for chlorinated wastewater ranged from 0.01 mg/L to 0.09 mg/L and averaged 0.04 mg/L as total residual chlorine. The 96-hour LC_{50} of chlorine dioxide disinfected wastewater could not be related to the chlorine dioxide residual. However, chlorine dioxide doses greater than 1 mg/L produced effluents which were acutely lethal in 96 hours. Indirect comparisons between the two disinfection processes indicated that chlorinated wastewater was more toxic than wastewater disinfected with chlorine dioxide. Both chlorine and chlorine dioxide disinfected effluents were non-acutely lethal following chemical dechlorination with sodium sulphite or 24 hours storage.

This report presents data on the acute lethality. Information relating to the cost and effectiveness of the various disinfectants is presented in a separate report.

RESUME

L'inquiétude grandissante qu'inspirent les dangers de la chloration des eaux usées urbaines pour l'environnement justifie la présente étude sur la toxicité aiguë d'autres méthodes de désinfection. Des contrôles biologiques réalisés en conditions dynamiques nous ont permis de comparer la toxicité aiguë, pour des truites arc-en-ciel, après 96 heures, d'eaux usées urbaines ayant subi un traitement secondaire. Les essais ont eu lieu avant et après la désinfection au chlore, au dioxyde de chlore, à l'ozone et au rayonnement ultra-violet. L'addition de sulfite de sodium neutre ou l'entreposage pendant 24 h permettait la déchloration.

Pendant les huit mois d'essais, les eaux usées urbaines non désinfectées n'ont montré aucune toxicité aiguë, 15 fois sur 17; les eaux désinfectées à l'ozone elles, aucune, 5 fois sur 6. La mortalité observée dans ces deux types d'eaux résultait directement des défaillances mécaniques des systèmes de traitement. Les eaux désinfectées au rayonnement ultra-violet n'avaient, elles non plus, aucune toxicité aiguë.

Le LC_{50}*, après 96 h, des eaux usées chlorées variait de 0.01 à 0.09 mg/L, et la teneur en chlore résiduel total s'élevait en moyenne à 0.04 mg/L. Le LC_{50}, après 96 h, des eaux usées désinfectées au dioxyde de chlore n'a pu être attribué à la présence de ClO_2 résiduel. Toutefois, des concentrations supérieures à 1 mg/L ont donné des effluents toxiques après 96 h. Une comparaison indirecte des deux procédés de désinfection a montré que les eaux usées chlorées se révélaient plus toxiques que celles désinfectées au dioxyde de chlore. Les deux types d'effluents cessaient d'execer une toxicité aiguë après une déchloration chimique au sulfite de sodium neutre ou un entreposage de 24 h.

Le présent rapport contient les données sur la toxicité aiguë. Les renseignements portant sur les coûts et l'efficacité des divers types de désinfectants apparaissent dans un autre rapport.

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*Lethal Concentration 50%: taux de concentration causant 50 p. 100 de décès au sein d'une population exposée à l'effluent toxique pendant une période déterminée (96 h).

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CONCLUSIONS

A program was carried out to determine the acute lethality of secondary treated municipal wastewater, following disinfection with chlorine, chlorine dioxide, ozone and ultraviolet light, and to determine if chlorine and chlorine dioxide disinfected wastewater could be rendered non-acutely lethal by sodium sulphite addition or 24 hours storage. In accordance with these objectives, the following conclusions were reached:

1. Chlorine disinfected wastewater was acutely lethal to rainbow trout. The 96-hour LC_{50} averaged 0.04 mg/L as total residual chlorine and ranged from 0.01 mg/L to 0.09 mg/L. The wide range in LC_{50}'s reflected the difficulty of maintaining constant chlorine residuals in the wastewater.
2. Dechlorination by sodium sulphite addition or by 24 hours storage effectively removed the acute lethality from chlorinated wastewater. A sulphite residual of up to 10 mg/L in the wastewater had to be maintained to compensate for fluctuating chlorine residuals.
3. Chlorine dioxide disinfected wastewater was acutely lethal to rainbow trout when the chlorine dioxide dose exceeded 1 mg/L.
4. Sodium sulphite addition or 24 hours storage effectively removed the acute lethality associated with chlorine dioxide disinfection.
5. Wastewater disinfected with chlorine dioxide was one third to one quarter as toxic as chlorinated wastewater having the same degree of disinfection. This may be due to the rapid decay of the chlorine dioxide residual.
6. Ozone disinfection produced non-acutely lethal effluent when the disinfection process was operating normally. The non-acutely lethal results observed in this study were due to the rapid decay of the ozone residual.

7. Disinfection with ultraviolet light produced a non-acutely lethal effluent.
8. Secondary treated, non-disinfected municipal wastewater was non-acutely lethal to rainbow trout in 15 of 17 trials.

RECOMMENDATIONS

1. Since chlorine and chlorine dioxide disinfected wastewaters are acutely lethal to rainbow trout, all sewage treatment plants employing chlorine or chlorine dioxide disinfection should reduce the residuals to non-detectable levels by chemical addition or natural decay prior to discharge.
2. In terms of fish toxicity, disinfection with ozone or ultraviolet light are the best environmentally compatible alternatives to chlorine disinfection. Evaluation of ozone and ultraviolet light on full scale treatment systems is needed to determine which of the two disinfectants is the most acceptable.

1 INTRODUCTION

1.1 Scope of Study

This study was part of a larger cooperative program between the Environmental Protection Service (Department of the Environment) and the Ontario Ministry of the Environment which investigated disinfection of a secondary municipal effluent. Aims of the study were to determine the disinfection efficiency and acute lethality to fish associated with chlorine, chlorine dioxide, and ozone. A concurrent study using ultraviolet light disinfection was carried out by personnel of the Environmental Management Service, Canada Centre for Inland Waters, Burlington, Ontario.

This report contains bioassay data on the acute lethality of wastewater following disinfection with chlorine dioxide, ozone and ultraviolet light, plus the acute lethality of dechlorinated wastewater. Information on the disinfection efficiency and costs of the various disinfection alternatives considered in the main study has been reported by Tonelli et al (1978). Information on the ultraviolet light study has been reported by Oliver and Carey (1976).

1.2 Background

The use of chlorine for disinfecting water and wastewater presents a dilemma to agencies responsible for public health and environmental protection. On one hand, sufficient disinfection is necessary to reduce the health hazard associated with pathogenic microorganisms, but on the other hand, the concentration of chlorine necessary to achieve disinfection is known to cause significant adverse effects to both fish and aquatic invertebrates.

Arthur et al (1975) reported that the seven-day LC_{50}'s for fish and invertebrates ranged from 0.08 mg/L to greater than 0.81 mg/L of chlorine depending upon the species tested. The LC_{50} is the concentration of toxicant which is lethal to 50% of the test animals within a specified time; usually 96 hours or less. Longer sub-lethal bioassays produced measurable adverse effects on minnows (*Pimephales promelas*) and *Daphnia magna* at residual chlorine concentrations as low as 0.042 mg/L and 0.018 mg/L, respectively (Arthur et al, 1975).

Lethal levels of chlorine, estimated from laboratory bioassays, have been verified by field studies on the impact of chlorinated effluents on receiving waters (Esvelt et al, 1973; Brown and Beck, 1972; Tsai, 1973; Servizi and Martens, 1974). The Michigan Bureau of Water Management (1971) found that stream reaches within 1.3 km (0.8 miles) of a chlorinated sewage outfall were lethal to rainbow trout. The estimated 96-hour LC_{50}'s were 0.014 mg/L and 0.029 mg/L of residual chlorine for the two downstream sites studied. No mortality occurred at the same sites when chlorination was temporarily interrupted. Brungs (1973) extensively reviewed the lethal and sub-lethal effects of chlorine disinfected wastewaters on fish and concluded that prolonged exposure to chlorine concentrations greater than 0.01 mg/L would be lethal to salmon and trout. He recommended that total residual chlorine concentrations not exceed 0.002 mg/L in receiving waters to ensure protection for most aquatic organisms continuously exposed to chlorinated discharges.

There are basically two means of achieving the degree of environmental protection suggested by Brungs (1973); the first is to reduce the impact of chlorine by following disinfection with a dechlorination stage. To date, sodium bisulphite (Esvelt et al, 1973; Brown and Beck, 1972), sulphur dioxide (Arthur, 1971-1972; Martens and Servizi, 1975) and sodium thiosulphate (Zillich, 1972) have been reported as successful dechlorinating agents. In addition, many authors have recommended natural decay as a method of dechlorination for small municipalities (Martens and Servizi, 1975; Esvelt et al, 1973; Tsai, 1973).

The second approach is to provide an environmentally acceptable alternative to chlorine disinfection. Possible replacements include: ozone (Arthur et al, 1975; Nebel et al, 1973; Ward et al, 1976); ultraviolet light (Dean, 1974; Oliver and Carey, 1976); bromine chloride (Arthur et al, 1975; Ward et al, 1976); and chlorine dioxide. No information was found at the time of this study on the lethality of chlorine dioxide disinfected wastewater, or on the removal of chlorine dioxide residual by chemical addition or natural decay.

2 OBJECTIVES

The objectives of this project were:

1. To compare the acute lethality of secondary treated wastewater disinfected with chlorine, chlorine dioxide, ozone or ultraviolet light.
2. To determine the acute lethality of chlorine and chlorine dioxide disinfected wastewater following sodium sulphite addition.
3. To determine the effect of storage on the acute lethality of chlorine and chlorine dioxide disinfected wastewater.

3 PROCEDURES

3.1 Study Site

The study was carried out at the Ontario Ministry of the Environment's Experimental Facility (OEF) located in Brampton, Ontario. The OEF is a 936 m^3/h (5 Imgd) conventional activated sludge plant which is currently used by the Ministry for research and operator training.

The sewage treatment plant consists of four treatment systems; each with a primary clarifier, aeration basin and secondary clarifier. Raw sewage from the municipality of Brampton enters a common headwork for comminution and grit removal prior to treatment, and final effluent from the plant is discharged to a much larger water pollution control plant.

3.2 Experimental Program

The experimental program was separated into the two phases described in Tables 1 and 2. Emphasis during the first phase was placed on the disinfection efficiency and acute lethality of chlorine, chlorine dioxide and ultraviolet light, plus the lethality associated with chemical dechlorination. Nominal chlorine residual during this phase ranged from 0.5 mg/L to 2.5 mg/L at a nominal chlorine dioxide dose of 0.8 mg/L to 4.0 mg/L. A control test with non-disinfected effluent was used to differentiate between mortality resulting from the disinfecting agents and from the treated municipal wastewater. Additional bioassays were performed to determine if sodium sulphite, the dechlorinating agent, and sodium chlorite, an interaction product of chlorine dioxide disinfection, were acutely lethal to rainbow trout.

The disinfection efficiency and acute lethality of ozone and chlorine dioxide disinfected wastewater were determined during the second phase of the study (Table 2). Nominal ozone and chlorine dioxide doses ranged from 10 to 25 mg/L and 3.0 to 6.6 mg/L, respectively. Bioassays were also conducted to determine the lethal effects of dechlorination by sodium sulphite addition and natural decay. Again, each test period included a non-disinfected effluent bioassay as the control.

TABLE I. EXPERIMENTAL PROGRAM - PHASE I: FEBRUARY TO MAY, 1975

Date	Treatment				
	Non-Disinfected	Chlorine Nominal Residual (mg/L)	Dechlorinated with Na_2SO_3	Chlorine Dioxide Nominal Dose (mg/L)	Ultraviolet Light
Feb. 3 - Feb. 7	X	0.5	X		X
Feb. 11 - Feb. 15	X	1.5	X		X
Feb. 17 - Feb. 21	X	0.5	X		
Mar. 3 - Mar. 7	X	2.5	X	4.0	
Mar. 10 - Mar. 14	X	2.5	X	4.0	
Mar. 17 - Mar. 21	X	0.5		0.8	
Apr. 7 - Apr. 11	X, X + 10 mg/L Na_2SO_3	1.5		2.6	
Apr. 14 - Apr. 18*	X, X + 10 mg/L $NaClO_2$	0.5		1.3	
Apr. 21 - Apr. 25	X, X + 10 mg/L Na_2SO_3	1.0		1.5	
Apr. 28 - May 2	X	1.0	X	1.4	

X - Indicates that a bioassay test was conducted for this treatment.
* - Control mortality - all bioassays on these dates have been excluded from this report.

TABLE 2. EXPERIMENTAL PROGRAM - PHASE II: SEPTEMBER TO NOVEMBER, 1975

Date	Treatment						
	Non-Disinfected	Chlorine Nominal Residual (mg/L)	Dechlorinated by Decay	Ozone Nominal Dose (mg/L)	Chlorine Dioxide Nominal Dose (mg/L)	Dechlorinated	
						Decay	Na_2SO_3
Sept. 15 - Sept. 19	X			10 - 12	3.0		X
Sept. 22 - Sept. 26**	X	1.0	X	12 - 13			
Sept. 29 - Oct. 3	X			13	3.5		X
Oct. 6 - Oct. 10*	X			13 - 15	4.6	X	
Oct. 21 - Oct. 25	X	1.0	X	13 - 25			
Oct. 27 - Oct. 31	X			13 - 25	6.6	X	
Nov. 3 - Nov. 7	X			15 - 25	6.3	X	

X - Indicates that a bioassay test was conducted for this treatment.
* - Control mortality - all bioassays on these dates have been excluded from this report.
** - Chlorinated and dechlorinated bioassays excluded due to process upsets.

3.3 Wastewater Treatment

The bioassay data were collected during periods when the treatment system was producing effluents with little or no nitrification. Raw wastewater and final effluent characteristics for the bioassay periods are reported in Tables 3 and 4, respectively.

TABLE 3. RAW WASTEWATER CHARACTERISTICS*

Parameter	Feb. 3 to Feb. 26		Feb. 27 to May 4		Sept. 1 to Nov. 7	
	Average	Range	Average	Range	Average	Range
BOD_5 (mg/L)	186	75 - 340	258	46 - 530	243	144 - 377
COD (mg/L)	688	250 - 1 660	517	130 - 1 176	694	336 - 1 286
SS (mg/L)	280	14 - 1 080	293	40 - 950	475	186 - 1 860
TKN (mg/L)	37	15 - 65	32	18 - 60	41.8	27 - 50
P (mg/L)	7.1	4.5 - 11	7.0	3.5 - 14	8.6	6.8 - 12.7

* Raw wastewater characteristics supplied by Mr. F.A. Tonelli, Ontario Ministry of the Environment.

TABLE 4. NON-DISINFECTED EFFLUENT CHARACTERISTICS*

Parameter	Feb. 3 to Feb. 26		Feb. 27 to May 4		Sept. 1 to Nov. 7	
	Average	Range	Average	Range	Average	Range
F/M	0.2	0.1 - 0.3	0.3	0.1 - 0.7	0.3	0.1 - 0.6
Temperature	11.4	10 - 12.5	12.6	11 - 14	20.5	18 - 25
BOD_5 (mg/L)	14.6	5 - 26	16.7	3 - 50	23.1	7.5 - 179
COD (mg/L)	68	30 - 100	67	20 - 140	75.4	20 - 278
SS (mg/L)	14	10 - 25	18	2 - 130	30.6	5.5 - 198
NH_3-N (mg/L)	10.3	0.8 - 24	14	0.8 - 24	9.4	0.2 - 19
NO_2-N (mg/L)	0.7	0.2 - 5.8	0.5	0.1 - 1.8	2.8	0.1 - 13
NO_3-N (mg/L)	7.4	1.5 - 12.8	2.1	0.2 - 7.4	3.5	0.2 - 10
P (mg/L)	4.0	2.2 - 7	4.4	2.2 - 12	4.1	2.9 - 9.1
Colour (Hazen Units)	60.3	40 - 85	63.1	30 - 100	80.1	40 - 120

* Non-disinfected effluent characteristics supplied by Mr. F.A. Tonelli, Ontario Ministry of the Environment.

During the first month of the program (February 3 to 26), the F/M ratio averaged 0.2 and ranged from 0.1 to 0.3. The effluent was partially nitrified for all of this period with a gradual reduction in nitrate concentration from 12.8 mg/L to 1.5 mg/L. Nitrite concentrations were also reduced from 5.8 mg/L to 0.2 mg/L indicating an upset in the nitrification process.

From February 27 through May 4 the treatment plant was operated at an average F/M ratio of 0.3 with a range from 0.1 to 0.7. The low nitrate and nitrite concentrations reported in Table 4 indicate no nitrification throughout this period.

The F/M ratio during the second phase (September 1 to November 7) averaged 0.3 and ranged from 0.1 to 0.6. Some nitrification occurred during the early part of September (nitrate concentrations ranged from 3 mg/L to 5 mg/L), however, there was no nitrification between October 6 to 14 and varying degrees of nitrification from October 14 to November 7. Nitrite concentrations gradually increased from 0.1 mg/L to 13 mg/L, indicating periods of instability in the nitrification process.

3.4 Effluent Distribution

Approximately 131 m^3/d (20 Igpm) of non-disinfected effluent was diverted to the bioassay trailer to serve as the control for each bioassay test. Another portion of the final effluent from the activated sludge plant was pumped to the experimental area for disinfection by either chlorine, chlorine dioxide, ozone or ultraviolet light. Following disinfection, the effluent was pumped directly to the bioassay trailer for testing. Portions of the chlorine and chlorine dioxide disinfected effluents were diverted to the chemical dechlorinator and the off-line storage tanks. Effluents from these two treatments were subsequently pumped to the bioassay trailer for toxicity testing. The flow distribution is illustrated in Figure 1.

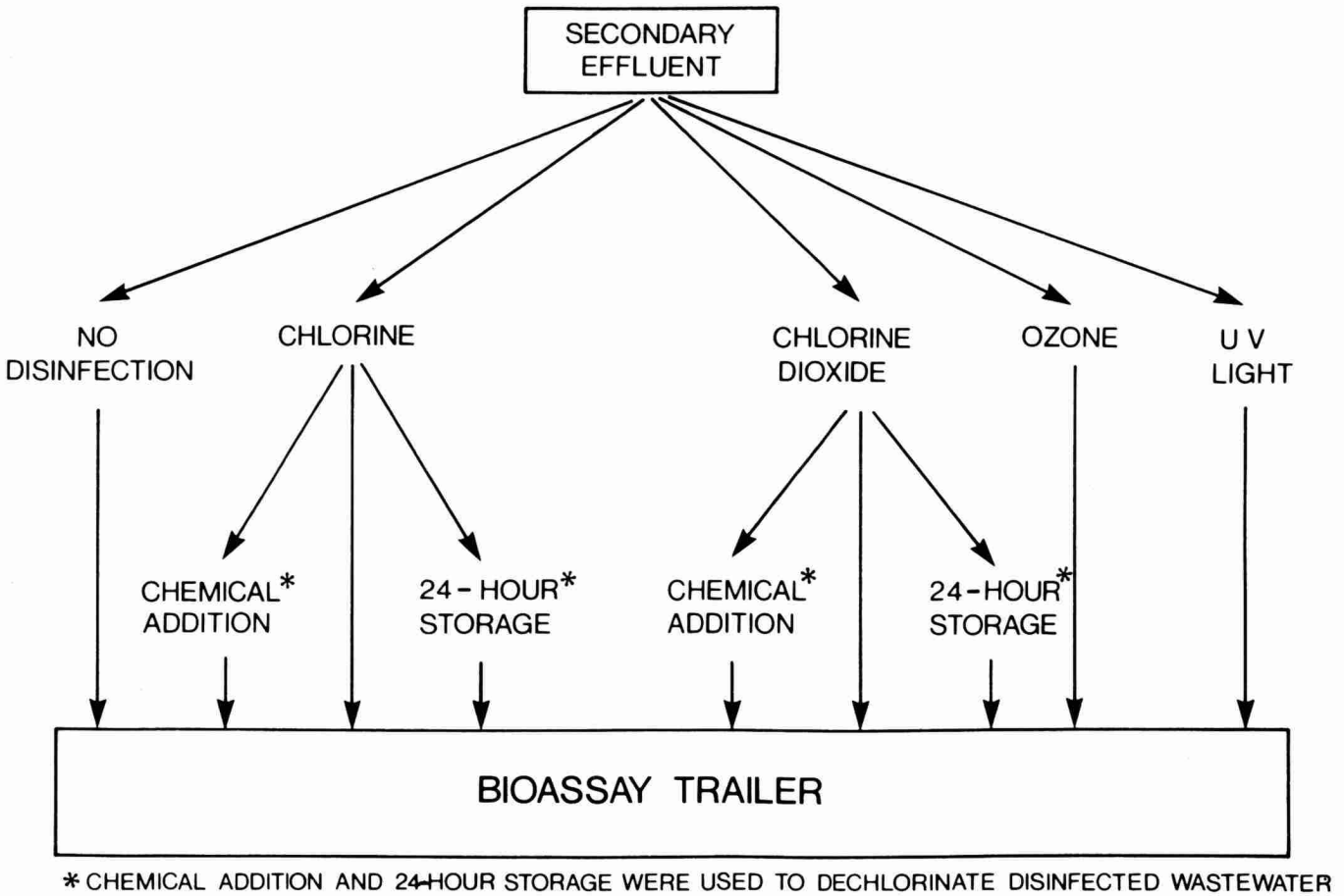

FIGURE 1. WASTEWATER DISTRIBUTION SYSTEM

3.5 Disinfection Systems

3.5.1 Chlorination

The chlorine disinfection system had a nominal capacity of 655 m^3/d (100 Igpm) at 30 minutes contact time. Chlorine gas was introduced to the effluent by a vacuum chlorinator and ejector through a concentrate mixing stage and into a contact chamber providing 15 to 45 minutes of detention time. Nominal total chlorine residuals during the study ranged from 0.5 mg/L to 2.5 mg/L (Tables 1 and 2).

3.5.2 Chlorine dioxide

Chlorine dioxide was prepared as a concentrated solution of up to 11% strength by the reaction of sodium chlorite and hydrochloric acid. In this study, an excess of 300% hydrochloric acid was used in a continuous reaction.

This reaction is not economic at large scale, but is useful in pilot plant operations since co-production of chlorine is minimal and gaseous chlorine or gaseous chlorine dioxide need not be handled. The chlorine dioxide disinfection system had a nominal capacity of 229 m^3/d (35 Igpm). The contact tank provided approximately 20 minutes detention time. Nominal chlorine dioxide dose during the study was varied from 0.8 mg/L to 6.6 mg/L (Tables 1 and 2).

3.5.3 Dechlorination systems

3.5.3.1 Natural decay. Chlorine and chlorine dioxide disinfected wastewaters were dechlorinated by natural decay during a 24-hour storage period. Following disinfection, the wastewater was pumped into one of two rubber-lined steel tanks where it was circulated slowly to prevent settling of solids and to help maintain the dissolved oxygen concentration at 5 mg/L to 7 mg/L. Chlorine and chlorine dioxide residuals were analyzed regularly by amperometric titration and dechlorinated effluent was pumped to the bioassay trailer when the residuals had decayed to undetectable levels.

3.5.3.2 Chemical dechlorination. Dechlorination by chemical addition was accomplished by metering a sodium sulphite solution to the wastewater in a small contact tank which provided approximately 15 minutes contact time. The system produced 131 m^3/d (20 Igpm) of dechlorinated effluent containing from 1 mg/L to 10 mg/L of residual sulphite. Average sulphite residuals for each bioassay period are reported in Table 5.

TABLE 5. SULPHITE RESIDUALS IN WASTEWATER AFTER SODIUM SULPHITE ADDITION*

Date	Sulphite Residual following Chemical Addition (mg/L)*
Feb. 3 - Feb. 7	1.4
Feb. 11 - Feb. 15	8.7
Feb. 17 - Feb. 21	9.2
Mar. 3 - Mar. 7	9.5
Mar. 10 - Mar. 14	1.0
Apr. 28 - May 2	9.6
Sept. 15 - Sept. 19	2.8**
Sept. 29 - Oct. 3	1.7**

* All values have been averaged over the four-day bioassay period.
** Chlorine dioxide disinfection.

Chlorine dioxide residuals were removed by adding concentrated sodium sulphite to the wastewater followed by a 30-minute detention time in a baffled contact tank. The chemically dechlorinated effluents contained 2.8 mg/L and 1.7 mg/L of residual sulphite (Table 5).

3.5.4 Ozone

Ozone was produced from oxygen in a Trailigaz "Ozo Lab" Ozonator with a nominal capacity of 16 g O_3/h. The contact chamber consisted of two polyvinyl chloride (PVC) columns connected in series. Wastewater entering the top of the first column, discharged at the bottom then entered the bottom of the second column and discharged at the top.

Ozone was introduced at the bottom of each column so that the ozone wastewater interaction was countercurrent in the first column and co-current in the second. Total contact time in the two columns was approximately 18 minutes at a flow rate of 26 m^3/d (4 Igpm). The ozone dose, which varied from 10 mg/L to 25 mg/L ozone during the study, is reported in Table 2.

3.5.5 Ultraviolet light

The ultraviolet light system consisted of a 56-litre (12.4 Ig) container equipped with a flat weir and two baffles to prevent short circuiting. Low pressure mercury lights were suspended directly above the surface so that disinfection occurred as the effluent passed over the weir in a thin film. Flow of ultraviolet disinfected effluent ranged from 7 to 131 m^3/d (1.1 to 20 Igpm) and ultraviolet dose was varied from 5×10^{-8} to 6×10^{-8} einsteins/mL. A detailed description of the apparatus and the disinfection efficiency has been reported by Oliver and Carey (1976).

3.6 Bioassays

Disinfected and non-disinfected effluents were tested for acute lethality in a mobile bioassay laboratory provided by the Wastewater Technology Centre.

The bioassay laboratory is a 11.0 x 3.6 m custom designed trailer complete with three, 900-litre (200 Imperial gallons), fish holding tanks, six Mount-Brungs (Mount and Brungs, 1967) continuous flow diluters, temperature control for both water and effluent, plus sufficient bench space for chemical analyses.

The toxicity tests were 96-hour, continuous flow, on-line bioassays. A total of five effluents, including the non-disinfected effluent which served as a control for each operating period were tested simultaneously. Lag time between the disinfection chambers and the bioassay laboratory, with the exception of effluents dechlorinated by decay, was approximately 20 minutes.

Bioassay procedures were similar to those described in Standard Methods (1971). Each bioassay consisted of five effluent concentrations; (100%, 50%, 25%, 12.5%, 6.2%) plus a control, with 10 juvenile rainbow trout (Salmo gairdneri) per concentration. Each test concentration was aerated at 100 cc/min with a small pore airstone. Initial attempts were made to aerate the effluents in the head tanks, but mortality occurred when flow interruptions caused the dissolved oxygen in the test containers to drop below 2 mg/L. All bioassays were carried out at 15 ± 1°C.

The continuous flow diluters supplied each test concentration with 166 mL of test solution per minute and provided a 90% molecular change of test solution every 4.5 hours. Effluent volume to fish weight ratio ranged from 4.6 L/g/d to 36.0 L/g/d. Observations on mortality were made at 0.25, 0.5, one, two, four and eight hours from the start of the bioassay and once every two hours between 0800 and 2300 hours on subsequent days. In addition to recording mortalities, analyses for ozone, total residual chlorine, sulphite, ammonia, pH, temperature and dissolved oxygen were made twice daily on effluents in the feed lines, the head tanks and the 100% test concentrations. If mortality occurred in the 100% concentrations, lower concentrations were also analyzed.

4 RESULTS AND DISCUSSION

4.1 Non-Disinfected Effluent

Non-disinfected effluent was non-acutely lethal in 15 of the 17 bioassays. The two lethal effluents occurred during April 14 to 18 and October 6 to 10 bioassay periods and were characterized by ammonia concentrations of approximately 18 mg/L. Ammonia is reported to be acutely lethal in the un-ionized form (NH_3) at concentrations exceeding 0.2 mg/L (EIFAC, 1973) and the degree of ionization is dependent upon pH and temperature. Trussell (1972) calculated the percent of NH_3 present in ammonia solutions at various pH's and temperatures, and found approximately 1.7% of the total ammonia was available as NH_3 during the two lethal bioassays. This corresponds to 0.3 mg/L of NH_3 and could be responsible for the mortality observed in the two trials. Bioassays with disinfected effluents conducted during the two periods of control mortality have not been included in this report.

4.2 Chlorinated Effluent

Chlorine residuals in the effluent immediately following the disinfection chamber closely approximated the nominal chlorine residuals reported in Tables 1 and 2. However, the total residual chlorine concentrations in the bioassay tests were reduced by a factor of two to four as a result of the 20-minute lag time between the disinfection chamber and the bioassay trailer, and from agitation of the effluent in the diluter and aeration in the test vessels. Although the total residual chlorine was reduced in the bioassay vessels, all chlorinated effluents were acutely lethal to rainbow trout. The calculated 96-hour LC_{50}'s based on the mean chlorine residual in the bioassay during the four-day test period ranged from 0.01 mg/L to 0.09 mg/L with an average of 0.04 mg/L (Table 6). This agrees with Ward et al (1976) who reported the 96-hour LC_{50} for rainbow trout to be 0.069 mg/L.

TABLE 6. THE 96-HOUR LC_{50}'S FOR CHLORINE DISINFECTED EFFLUENT

Date	Nominal Chlorine Residual (mg/L)	Chlorine Concentration			96-h LC_{50} (mg/L)	95% Confidence Limits (mg/L)
		Immediately following Contact Chamber[2] (mg/L)	Bioassay Test[2] (mg/L)	Range[3] (mg/L)		
Feb. 3 - Feb. 7	0.5	0.84	0.32	0.0 - 1.10	0.09	0.07 - 0.12
Feb. 11 - Feb. 15	1.5	1.23	0.32	0.10 - 0.65	0.05	0.03 - 0.06
Feb. 17 - Feb. 21	0.5	0.50	0.03	0.0 - 0.09	0.01[1]	0.01 - 0.02
Mar. 3 - Mar. 7	2.5	1.95	0.86	0.14 - 1.80	0.04	0.03 - 0.05
Mar. 10 - Mar. 14	2.5	2.02	0.53	0.0 - 1.00	0.05[1]	0.03 - 0.07
Mar. 17 - Mar. 21	0.5	0.63	0.30	0.02 - 0.81	0.08	0.06 - 0.11
Apr. 7 - Apr. 11	1.5	1.32	0.99	0.24 - 1.62	0.04	0.03 - 0.05
Apr. 14 - Apr. 18	0.5	0.35	0.13	0.02 - 0.39	0.03	0.02 - 0.04
Apr. 21 - Apr. 25	1.0	0.81	0.20	0.0 - 0.51	0.04[1]	0.03 - 0.05
Apr. 28 - May 2	1.0	0.52	0.11	0.0 - 0.43	0.03	0.02 - 0.04
Sept. 22 - Sept. 26	1.0	0.88	0.35	0.20 - 0.50	0.03	0.02 - 0.03
Oct. 21 - Oct. 25	1.0	NA*	0.36	0.10 - 0.60	0.03	0.02 - 0.04

[1] 96-hour LC_{50} estimated from concentrations producing 0 and 100% response. In these instances, the 95% confidence limits are the 0 and 100% responses.
[2] Chlorine concentrations are the average of grab samples collected twice daily during the four-day bioassay.
[3] Refers to the observed range of grab samples and does not necessarily represent the maximum and minimum concentrations during the four-day period.
* Not analyzed.

The wide range of the 96-hour LC_{50}'s reported in Table 6 resulted from difficulty in maintaining a constant chlorine residual in the effluent. Fluctuations were due to changes in the chlorine demand of the effluent plus occasional mechanical failures. Since short term exposure to a high concentration of chlorine produces irreversible physiological damage (Brungs, 1973; Servizi and Martens, 1974) the LC_{50}'s reflect the response of the fish to the maximum residual during the bioassay rather than the mean. A more precise estimate of the LC_{50} would require installation of a continuous chlorine monitor-controller, capable of maintaining a constant residual in spite of the fluctuating chlorine demand of the effluent.

4.3 Dechlorinated Effluent

Dechlorination by the addition of sodium sulphite resulted in a non-acutely lethal effluent (Figure 2). These results support the findings of previous studies (Esvelt et al, 1973; Brown and Beck, 1972). There is no available explanation for the lethal dechlorinated effluent during trial 2, however, mortality could have resulted from incomplete dechlorination during periods of maximum chlorine residual or from a slug of very toxic material. This is supported by the observation that no mortality or equilibrium loss occurred in 100% effluent after 47 hours, yet three hours later, 50% of the fish had died. Although dechlorinated effluents contained sulphite residuals less than 10 mg/L (Table 5), it was unlikely that the sulphite was responsible for the mortality in trial 2 since two control bioassays with non-disinfected effluent containing 10 mg/L of sodium sulphite were non-acutely lethal (Table 7). Arthur et al (1975) reported the seven-day LC_{50} for sulphite to be 67 mg/L for fathead minnows (Pimephales promelas) and 10 mg/L for amphipods. The LC_{50} of sulphite had been reported as high as 3 200 mg/L (Sano, 1976), but the sub-lethal effects of continuous exposure to sodium sulphite are unknown and require investigation.

Chlorine disinfected effluent was non-acutely lethal after 24 hours storage (Figure 2). Although only one trial was attempted, the result supports the proposals by Tsai (1973) and Martens and Servizi (1975) that the lagooning of chlorinated wastewater might be a means of removing chlorine toxicity.

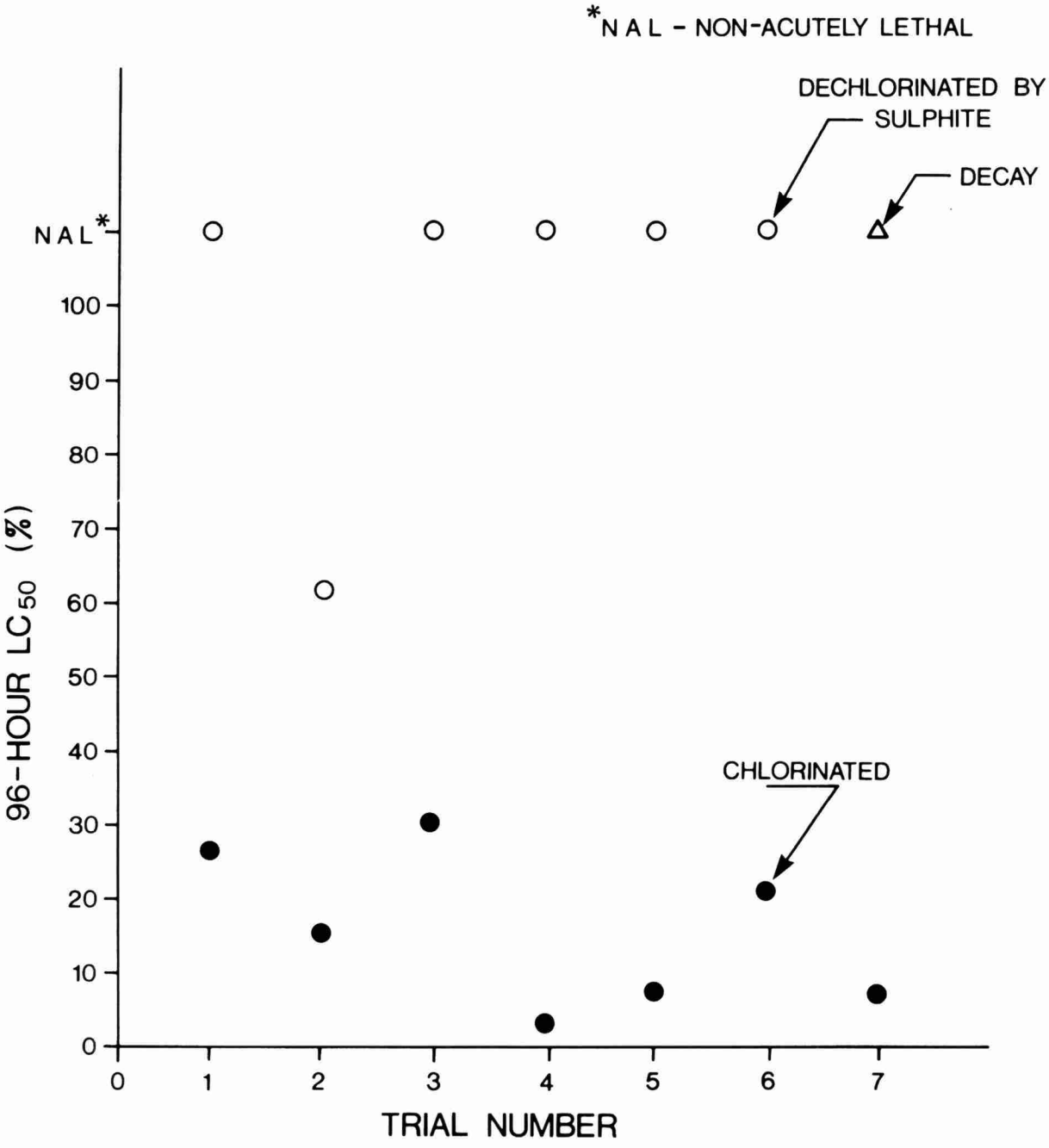

FIGURE 2. EFFECT OF CHEMICAL ADDITION AND NATURAL DECAY ON THE 96-HOUR LC_{50} OF CHLORINATED EFFLUENT

TABLE 7. ACUTE LETHALITY OF SODIUM SULPHITE AND SODIUM CHLORITE IN NON-DISINFECTED EFFLUENT

Date	Treatment	LC_{50}	Test Duration
Apr. 7 - Apr. 11	Non-disinfected effluent plus 10 mg/L Na_2SO_3	NAL*	77 hours
Apr. 14 - Apr. 18	Non-disinfected effluent plus 10 mg/L $NaClO_2$	NAL	96 hours
Apr. 21 - Apr. 25	Non-disinfected effluent plus 10 mg/L Na_2SO_3	NAL	96 hours
Apr. 28 - May 2	100 mg/L $NaClO_2$ plus dechlorinated water	NAL	96-hour static bioassay

* Non-acutely lethal. More than 50% of fish survived in 100% effluent.

4.4 Chlorine Dioxide Disinfected Effluent

Dean (1974) reported no recorded observations of toxicity when water was disinfected with chlorine dioxide. However, results from this study indicated that all chlorine dioxide disinfected effluents were acutely lethal. Ninety-six-hour LC_{50}'s ranged from 31 to 80% (Table 8).

TABLE 8. EFFECT OF NOMINAL CHLORINE DIOXIDE DOSE ON THE 96-HOUR LC_{50} OF CHLORINE DIOXIDE DISINFECTED EFFLUENT

Date	Nominal ClO_2 Dose (mg/L)	96-h LC_{50} (%)	95% Confidence Limits (%)
Mar. 3 - Mar. 7	4.0	35*	25, 50
Mar. 10 - Mar. 14	4.0	62*	50, 75
Mar. 17 - Mar. 21	0.8	80	69, 93
Apr. 7 - Apr. 11	2.6	35*	25, 50
Apr. 21 - Apr. 25	1.5	66	57, 77
Apr. 28 - May 2	1.4	35*	25, 50
Sept. 15 - Sept. 19	3.0	35*	25, 50
Sept. 29 - Oct. 3	3.5	35	28, 44
Oct. 27 - Oct. 31	6.6	35*	25, 50
Nov. 3 - Nov. 7	6.3	31	25, 38

* 96-hour LC_{50} interpolated from 0 and 100% responses. In these instances, 95% confidence limits have been replaced by the concentrations producing 0 and 100% mortality.

The results reported in Table 8 indicate that an increase in the nominal chlorine dioxide dose from 1.4 mg/L to 6.6 mg/L did not increase the lethality of the effluent. This may be due to a rapid loss of chlorine dioxide residual. Preliminary studies at the Wastewater Technology Centre indicated that in nitrified-denitrified wastewater, a dose of 6 mg/L produced residuals of 1.0 mg/L after 15 minutes and 0.12 mg/L after four hours (Figure 3).

At the beginning of the study, no satisfactory analytical technique was available to measure chlorine dioxide residuals and 96-hour LC_{50}'s were related to nominal chlorine dioxide dosage. To achieve continuity, this practice was continued throughout the study despite the fact that the problems of amperometric determination were later resolved by the Ministry of the Environment and Knechtel et al (1978) developed a successful colourimetric method for chlorine dioxide anlaysis.

Although chlorine dioxide residuals could not be precisely determined, the results suggest that mortality in the chlorine dioxide disinfected wastewater was due to, or induced by, the chlorine dioxide residual rather than the chlorite produced from the interaction of chlorine dioxide and wastewater. Thackeray (personal communication) reported the 96-hour LC_{50} for sodium chlorite to vary between 180 mg/L and 320 mg/L in static bioassays with rainbow trout and a control bioassay containing 10 mg/L of sodium chlorite in non-disinfected effluent was also non-acutely lethal (Table 7).

Although the lethal concentration of chlorine dioxide could not be determined, an estimate of its relative toxicity with respect to chlorine was possible by comparing the 96-hour LC_{50}'s associated with at least a 90% reduction in total coliforms (Table 9) (Tonelli et al, 1978). Effluent disinfected with chlorine dioxide was less toxic than effluent disinfected with chlorine and, at the same time, chlorine dioxide provided a consistently higher degree of disinfection than chlorine.

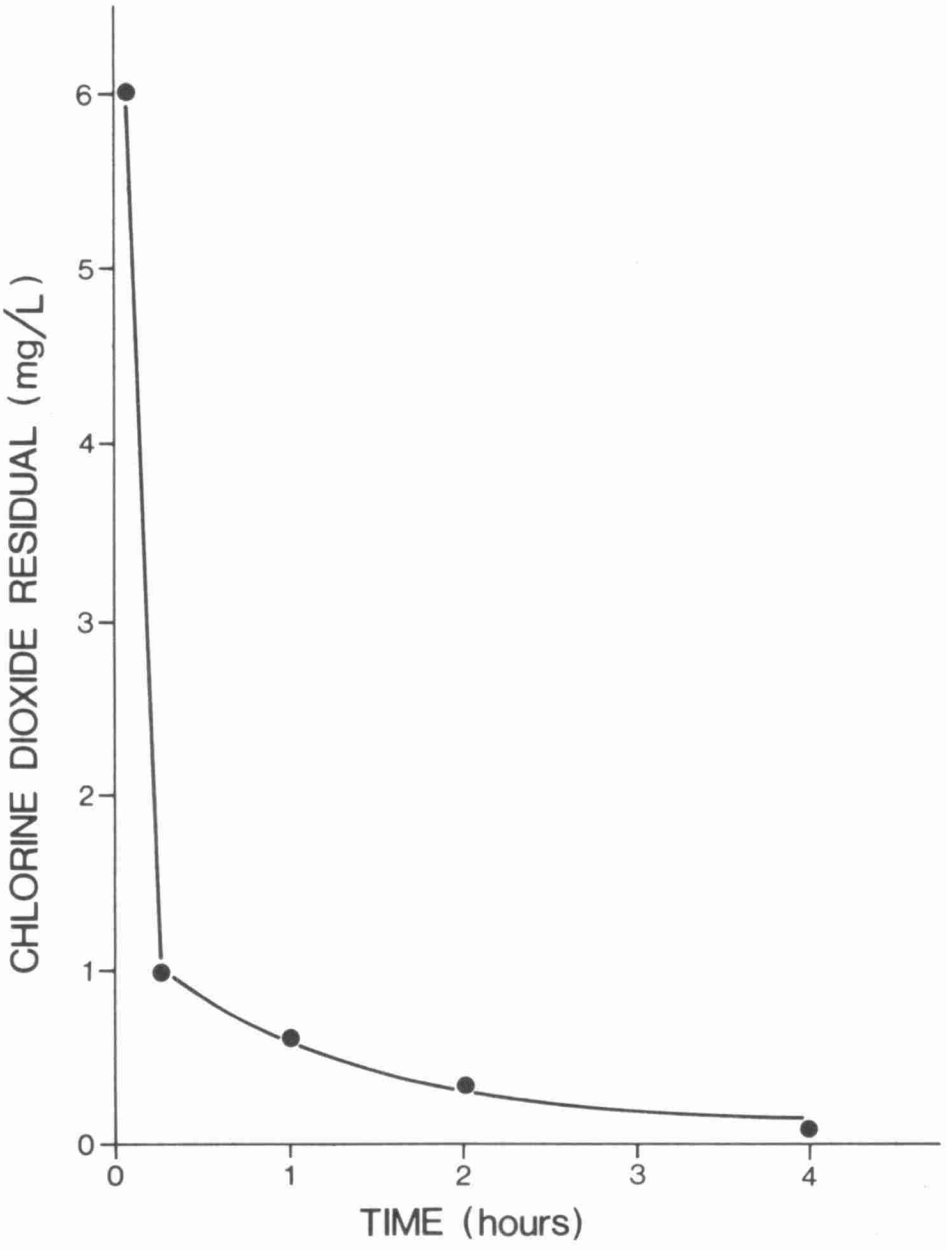

FIGURE 3. CHLORINE DIOXIDE RESIDUAL DECAY IN NITRIFIED-DENITRIFIED EFFLUENT

TABLE 9. COMPARISON OF 96-HOUR LC_{50}'S FOR CHLORINE AND CHLORINE DIOXIDE DISINFECTED EFFLUENT*

Chlorine		Chlorine Dioxide	
Total Coliform Reduction (%)	96-h LC_{50} (%)	Total Coliform Reduction (%)	96-h LC_{50} (%)
89.51	8.8	98.31	35
92.11	28.5	98.95	35
97.16	22.0	99.17	35
99.98	4.1	99.75	35
		99.93	31

* Disinfection data supplied by Mr. F.A. Tonelli, Ontario Ministry of the Environment.

4.5 Removal of Chlorine Dioxide Residual

Effluents disinfected with chlorine dioxide were non-acutely lethal after the addition of sodium sulphite (Figure 4). Mean sulphite concentrations during the two bioassays were 2.8 mg/L and 1.7 mg/L (Table 5). Similarly, storage for 24 hours reduced the toxicity in two trials (Figure 4).

4.6 Ozonated Effluent

Ozone disinfected wastewater was acutely lethal on four of the 15 tests. The mortality occurred when the flow rotameter measuring wastewater influent to the ozone columns partially plugged resulting in lower flows and higher ozone concentrations (residuals) than intended. Ward et al (1976) experienced similar difficulties and found it necessary to filter the effluent before ozonation. Invariably, this problem occurred during the night when both the ozonation system and the bioassays were unattended. When mortality was observed the following morning, the ozone residuals had disappeared, and, as a result, the lethal concentration of ozone could not be determined. Ward et al (1976) reported that ozone concentrations of 0.047 mg/L and 0.185 mg/L were non-lethal to goldfish and fathead minnows within 15 days. However, concentrations of 0.32 mg/L were lethal to lake trout fingerlings within five hours. Arthur et al (1975) found that ozone concentrations of 0.2 mg/L to 0.3 mg/L were

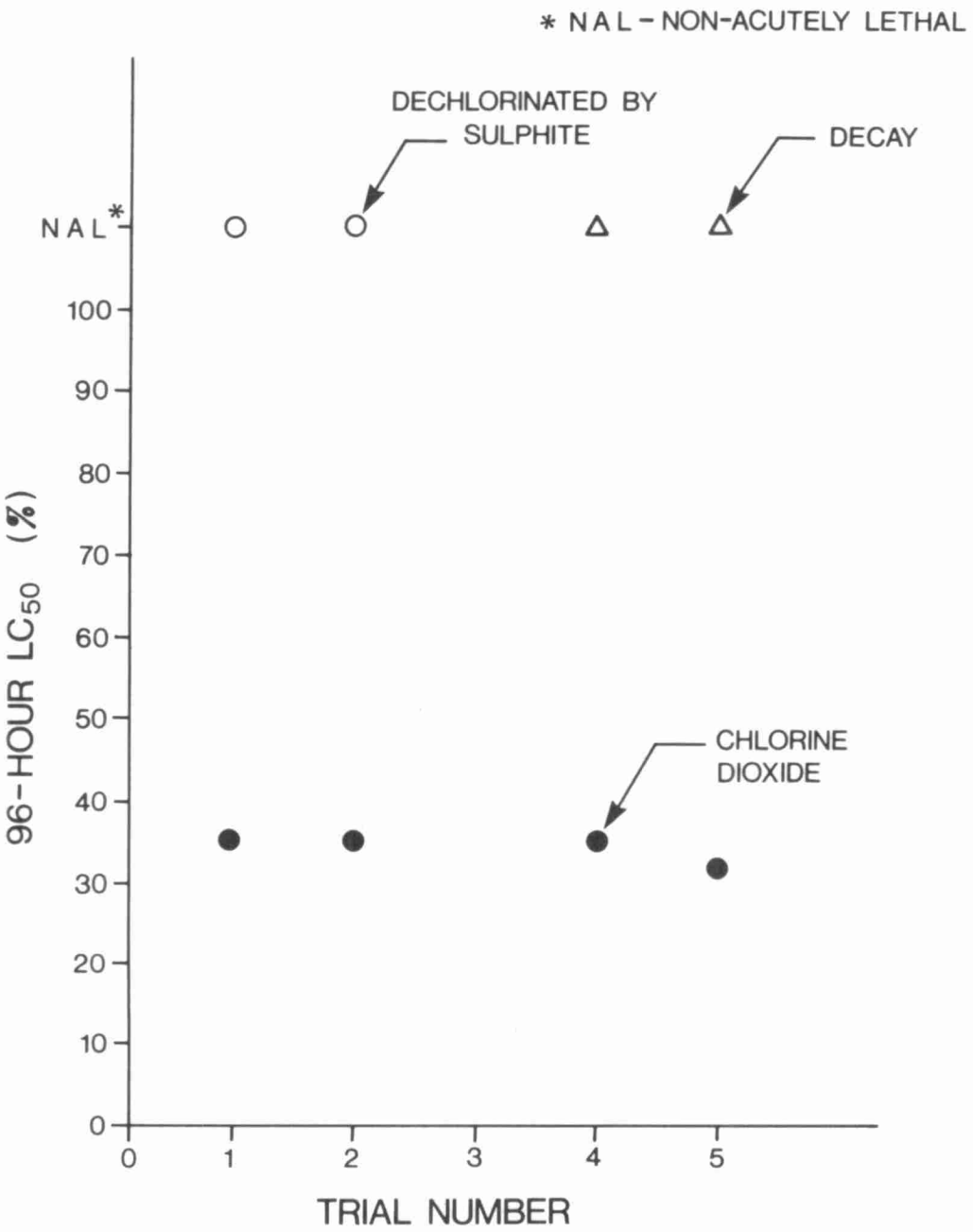

FIGURE 4. EFFECT OF CHEMICAL ADDITION AND NATURAL DECAY ON THE 96-HOUR LC_{50} OF CHLORINE DIOXIDE DISINFECTED EFFLUENT

lethal to fathead minnows, but special procedures were used to maintain the ozone residual in the effluent. During the present study, ozone residuals decayed to non-detectable levels within 15 minutes after disinfection with a 25 mg/L ozone dose. Arthur et al (1975) and Ward et al (1976) both reported no adverse effects on fish from long term exposure to ozone disinfected wastewater.

Since ozone residuals could not be detected during the routine bioassays, the degree of disinfection was used as a criterion to determine if the ozonation process was working effectively. Disinfection was considered to be effective if there was at least a 90% reduction in total coliforms. Six of the 15 tests provided this degree of disinfection and five of these six tests were not acutely lethal (Table 10). The single lethal bioassay resulted from increased contact time in the ozonation columns. Interestingly, the ozonated effluent was non-lethal during a period when the undisinfected effluent was lethal which suggests that the ozone wastewater interaction may provide some degree of toxicity reduction.

TABLE 10. NINETY-SIX-HOUR LC_{50}'S FOR NON-DISINFECTED AND OZONE DISINFECTED EFFLUENT

Date	Non-Disinfected LC_{50} (%)	Ozone Disinfected LC_{50} (%)
Sept. 15 - Sept. 19	NAL*	46 (37.9, 55.8)
Sept. 29 - Oct. 3	NAL	NAL
Oct. 6 - Oct. 10	81 (53.3, 100)	NAL
Oct. 21 - Oct. 25	NAL	NAL
Oct. 27 - Oct. 31	NAL	NAL
Nov. 3 - Nov. 7	NAL	NAL

* Non-acutely lethal. More than 50% of fish survived in 100% effluent.

4.7 Ultraviolet Disinfected Effluent

Two bioassays with ultraviolet disinfected wastewater were conducted. Both were non-acutely lethal. The degree of disinfection achieved during the two-week bioassay period exceeded 99% reduction in total coliforms. Unlike disinfection with chlorine, chlorine dioxide and ozone, ultraviolet disinfection is a photochemical process which prevents bacterial reproduction through alteration of the DNA (Oliver and Carey, 1976). As a result, there is no contamination of the effluent with lethal residuals nor a need for additional treatment to remove any toxicity which might be associated with these residuals.

5 GENERAL DISCUSSION

The 96-hour LC_{50}'s for chlorinated wastewater agree with published 96-hour LC_{50}'s for chlorinated fresh water and with LC_{50}'s determined by in situ bioassays below a wastewater outfall. Although chlorinated effluents were acutely lethal, they could be rendered non-acutely lethal by chemical dechlorination or by natural decay. Successful chemical dechlorination requires either a chlorine monitor-controller to maintain a constant chlorine residual, or continuous addition of excess sodium sulphite to remove the maximum chlorine residual. Removal of chlorine residual by storage was successful in one trial during the study. However, the storage time will almost certainly depend upon the initial chlorine concentration in the effluent. Even though 24 hours storage was sufficient to reduce the residuals in this study, Brown and Beck (1972) and Esvelt et al (1973) concluded that chlorine induced toxicity was not completely dissipated after three days storage.

Wastewater disinfected with chlorine dioxide was also acutely lethal to rainbow trout, but the effluent was less toxic than effluent receiving a lesser degree of disinfection with chlorine. The lethal concentration of chlorine dioxide could not be determined owing to interferences with the chlorine dioxide analysis. However, bioassay results indicate that wastewater disinfected with chlorine dioxide doses above 1 mg/L would be lethal to rainbow trout. Chlorine dioxide disinfected wastewater was non-lethal after sodium sulphite addition or 24 hours storage.

Wastewater disinfected with ozone was not acutely lethal within 96 hours to rainbow trout. This agrees with Arthur et al (1975) who concluded there was no measurable toxicity to aquatic life from either short or long term exposure to ozonated effluent. Long term, sub-lethal bioassays (Ward et al, 1976) produced no effect on the survival or reproduction of fathead minnows continuously exposed to ozone concentrations of less than 0.005 mg/L. In fact, it was concluded that "the ozonation process apparently eliminated or significantly reduced the inherent toxicity of the non-disinfected effluent". This view is supported by the single test in the present study in which a lethal, non-disinfected effluent was non-lethal after ozonation.

Ultraviolet light and ozone appear to be the most environmentally acceptable alternatives to chlorine disinfection. Both possess good disinfection capabilities and have no acutely lethal effect on fish.

Chlorine and chlorine dioxide disinfected effluents are both lethal to rainbow trout. Although storage could successfully remove the toxic residuals, the storage time required for complete decay would be site specific. Dechlorination by chemical addition could remove the acute lethality, but the system is not failsafe, and there is a potential risk associated with mechanical failures in the dechlorination stage.

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